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Effects of mutations and of ionophore on chlororespiration in *Chlamydomonas reinhardtii*

Pierre Bennoun

Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris, France

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It is shown that electron-transport elements of the photosynthetic chain located between plastoquinone and photosystem I centers are not involved in oxidation of plastoquinone in the chlororespiratory pathway. The ionophore dicyclohexyl-18-crown-6 is shown to induce a reduction of the plastoquinone pool in the dark. This is interpreted as indicating the existence of a coupling site in the chlororespiratory pathway between NAD(P)H and plastoquinone.

Chlamydomonas reinhardtii *Photosynthesis mutant* *Chlorophyll fluorescence* *Chlororespiration* *Ionophore* *Coupling site*

1. INTRODUCTION

We showed that a respiratory chain is present in the chloroplast [1]. This electron-transport chain from NAD(P)H to oxygen operates through the plastoquinone pool involved in the photosynthetic electron-transport chain. The thylakoid-bound NAD(P)H-plastoquinone oxidoreductase discovered in [2] is likely involved in the chlororespiratory pathway. Although specific for plastoquinone this enzyme is similar to the respiratory NADH dehydrogenase and consists of a flavoprotein with iron-sulfur centers. Here, we examine whether elements of the photosynthetic electron-transfer chain other than plastoquinone are involved in the chlororespiratory pathway. We also discuss the effect of the ionophore dicyclohexyl-18-crown-6 (crown) on chlororespiration.

2. MATERIALS AND METHODS

Chlamydomonas reinhardtii wild-type 137C and mutant strains were grown in TAP medium [3] under an illumination of 200 lux. Chlorophyll fluorescence kinetics were performed as in [4].

3. RESULTS AND DISCUSSION

We studied the rate of oxidation of the plastoquinone pool (PQ) in the dark in *Chlamydomonas* mutants which have impaired photosynthesis: we expect to observe a modification in the rate of oxidation of the PQ pool in mutants which are lacking an element common to the photosynthetic and respiratory chains. The results of these experiments are collected in table 1.

The absence of plastocyanin, cytochrome *f* or of the element missing in ac21 does not lead to an inhibition of the oxidation of the pool in the dark indicating that none of these components are directly involved in the oxidation of plastoquinone by the chlororespiratory chain. The absence of chloroplast ATPases did not affect markedly the oxidation of the PQ pool in the dark. It is not known what electron-transfer elements are present in the chlororespiratory pathway between plastoquinone and the oxidase. Several heme-proteins which have not been identified are revealed on SDS gels of thylakoid membranes by tetramethyl benzidine–H_2O_2 staining (P. Delepelaire, personal communication). Some of them could belong to the chlororespiratory pathway.

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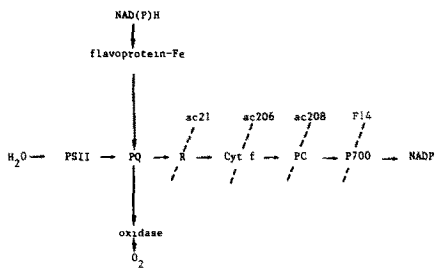
Table 1

Mutant strains	Element missing	A_o/Q	A_3/A_o
F14	PSI centers	9.8	0.49
ac 208	Plastocyanin	10	0.54
ac 206	Cytochrome *f*	10.6	0.75
ac 21	Rieske protein ?	11	0.74
F54.F14	PSI centers and ATPases	12.8	0.37

The mutant strains F14, ac 208, ac 206, ac 21 and F54.14 of *Chlamydomonas reinhardtii* were described in [5–7]. The ac 21 mutant has normal level of plastoquinone, cytochrome *f*, plastocyanin and PSI centers and is blocked in electron transfer between plastoquinone and cytochrome *f*. On this basis, it is probably deficient in the Rieske protein which is the only electron carrier known to be involved at this level of the photosynthetic electron-transfer chain. The ratio A_o/Q is obtained by measuring the area over the fluorescence rise in the absence (A_o) or in the presence (Q) of 10 μM DCMU in dark-adapted algae. The value of A_3 is given by the area over the fluorescence rise observed after reduction by light of the PQ pool followed by 3 s of reoxidation in the dark

The increase of the rate of oxidation of the PQ pool in the dark observed in mutants ac21 and ac206 (table 1) could indicate that the rate of input of electrons in the PQ pool is reduced in these mutants. However, anaerobiosis or CO treatment in the dark induce a reduction of the PQ pool in mutant ac21. This indicates that in this mutant, the input of electrons in the chlororespiratory chain is not blocked although the electron transfer in the photosynthetic chain is blocked after the PQ pool. The connection between the photosynthetic and chlororespiratory chains is depicted in scheme 1:



We showed in [1] that the inhibition of electron transfer in the chlororespiratory pathway led to a decrease in luminescence emission, indicating that this transfer contributes to the formation of an electrochemical gradient across the thylakoid membrane. We report here on the effect of an ionophore, dicyclohexyl-18-crown-6 (crown) which was shown to act in vivo (B. Diner, personal communication). We observed in the presence of crown a strong acceleration of the decay of the 515 nm change induced by a single short flash. The half-time of this decay goes from 600 ms in a mutant devoid of chloroplast ATPases to <1 ms in the same mutant treated with 1 mM crown. The permanent electrochemical gradient across the thylakoid membrane is thus collapsed in the presence of crown and the electron transfer in the chlororespiratory pathway becomes uncoupled. We see in fig.1 that the first effect of crown view in fluorescence is a partial reduction of the plastoquinone pool and a decrease of the initial fluorescence yield. The very same effect was observed in [1] after inhibiting chlororespiration by sodium azide which likely acts at the level of the chloroplast oxidase. The decrease of the initial fluorescence yield indicates the reactivation of some photosystem II centers that were inactivated

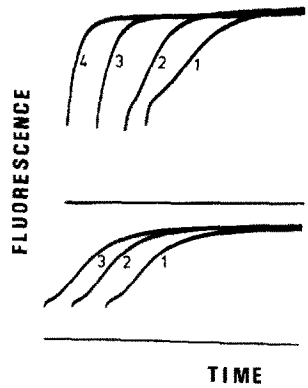


Fig.1. Upper: Effect of 1 mM crown on the fluorescence rise in dark-adapted cells of *Chlamydomonas* mutant F14 lacking photosystem I centers: (1) control; (2) 1 mM crown for 1 min. The pool size of electron acceptor to photosystem II is reduced to 70% of that in the control. (3) 1 mM crown for 2 min; (4) 1 mM Crown for 3 min. Lower: Effect of 1 mM crown on the fluorescence rise in dark-adapted broken spinach chloroplast: (1) control; (2) 1 mM crown for 1 min; (3) 1 mM crown for 3 min. Total sweep, 2 s.

by the permanent electrochemical gradient across the thylakoid membrane. In steady state conditions existing in the dark this membrane potential is composed predominantly of the pH gradient. A modulation of the activity of the photosystem II centers by the transmembrane electric field was observed in [8].

An increase in oxygen flash yield was observed in the presence of sodium azide [9]. A decrease in misses was observed when measuring the oxygen evolved during a flash sequence and a slowing down of the deactivation of the S2 and S3 states in the presence of sodium azide [9]. All these changes probably result from the decrease of the permanent electrochemical gradient consecutive to the inhibition of the chlororespiratory chain by sodium azide. It is important to note that the changes in the fluorescence rise in the presence of crown were observed in vivo but not in broken chloroplasts (fig.1). The reduction of the plastoquinone pool under uncoupling conditions (in the presence of crown) indicates that the rate of electron transfer in the chlororespiratory chain might be accelerated between NAD(P)H and plastoquinone rather than between plastoquinone and oxygen. That is, this effect may reveal the existence of a coupling site at the entrance of the chlororespiratory chain. This situation would be similar to that observed in other respiratory systems in which a coupling site exists between NADH and ubiquinone (complex 1).

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